[0006] Natural killer (NK) cells are a component of the innate immune system and make up approximately 15% of circulating lymphocytes. NK cells infiltrate virtually all tissues and were originally characterized by their ability to kill tumor cells effectively without the need for prior sensitization. Activated NK cells kill target cells by means similar to cytotoxic T cells – *i.e.*, via cytolytic granules that contain perforin and granzymes as well as via death receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN-gamma and chemokines that promote the recruitment of other leukocytes to the target tissue.

[0007] NK cells respond to signals through a variety of activating and inhibitory receptors on their surface. For example, when NK cells encounter healthy self-cells, their activity is inhibited through activation of the killer-cell immunoglobulin-like receptors (KIRs). Alternatively, when NK cells encounter foreign cells or cancer cells, they are activated via their activating receptors (*e.g.*, NKG2D, NCRs, DNAM1). NK cells are also activated by the constant region of some immunoglobulins through CD16 receptors on their surface. The overall sensitivity of NK cells to activation depends on the sum of stimulatory and inhibitory signals.

[0008] C-type lectin domain family 12 member A gene encodes a member of the C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily. Members of this family share a common protein fold and have diverse functions, such as cell adhesion, cell-cell signaling, glycoprotein turnover, and roles in inflammation and immune response. The protein encoded by this gene is a negative regulator of granulocyte and monocyte function. Human C-type lectin-like molecule-1 (CLL-1) also known as MICL or CLEC12A, is a type II transmembrane glycoprotein and member of the large family of C-type lectin-like receptors involved in immune regulation. CLL-1/CLEC12A is overexpressed in over 90% of acute myeloid leukemia patient on leukemic stem cells, but not on normal haematopoietic cells. The present invention provides multi-specific binding proteins that bind CLL-1/CLEC12A, and use of the proteins in treatment of cancer.

SUMMARY

[0009] The disclosure provides multi-specific binding proteins that bind to a tumor-associated antigen CLEC12A and to the NKG2D receptor and CD16 receptor on natural killer cells. Such proteins can engage more than one kind of NK activating receptor, and may block the binding of natural ligands to NKG2D. In certain examples, the proteins can agonize

NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various aspects and examples of the disclosure are described in further detail below.

[0010] Accordingly, one aspect of the disclosure provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds CLEC12A; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. The antigen-binding sites may each incorporate an antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.* arranged as in an antibody, or fused together to from an scFv), or one or more of the antigen-binding sites may be a single domain antibody, such as a V_HH antibody like a camelid antibody or a V_{NAR} antibody like those found in cartilaginous fish.

[0010a] In another aspect, the present disclosure refers to a formulation comprising the protein as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a cell comprising one or more nucleic acids encoding the protein as described herein. In a further aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of directly and/or indirectly enhancing tumor cell death comprising exposing the tumor cell and a natural killer cell to an effective amount of the protein as described herein.

[0010b] In another aspect, the present disclosure refers to the use of the protein as described herein or the formulation as described herein in the manufacture of a medicament for treating cancer. In a further aspect, the present disclosure refers to protein as described herein or the formulation as described herein for use in therapy.

[0011] The first antigen-binding site, which binds to NKG2D, in some examples, can incorporate a heavy chain variable domain related to SEQ ID NO:1, such as by having an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1, and/or incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:105), CDR2 (SEQ ID NO:106), and CDR3 (SEQ ID NO:107) sequences of SEQ ID NO:1. The heavy chain variable domain related to SEQ ID NO:1 can be coupled with a variety of light chain variable domains to form an NKG2D binding site. For example, the first antigen-binding site that incorporates a heavy chain variable domain related to SEQ ID NO:1 can further incorporate a light chain variable domain selected from any one of the sequences related to SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40. For example, the first antigen-binding site incorporates a heavy chain variable domain with amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID

NO:1 and a light chain variable domain with amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to any one of the sequences selected from SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.

[0012] Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:41 and a light chain variable domain related to SEQ ID NO:42. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:41, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:43), CDR2 (SEQ ID NO:44), and CDR3 (SEQ ID NO:45) sequences of SEQ ID NO:41. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:42, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:46), CDR2 (SEQ ID NO:47), and CDR3 (SEQ ID NO:48) sequences of SEQ ID NO:42.

[0013] In other examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:49 and a light chain variable domain related to SEQ ID NO:50. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:49, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:51), CDR2 (SEQ ID NO:52), and CDR3 (SEQ ID NO:53) sequences of SEQ ID NO:49. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:50, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:54), CDR2 (SEQ ID NO:55), and CDR3 (SEQ ID NO:56) sequences of SEQ ID NO:50.

[0014] Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:57 and a light chain variable domain related to SEQ ID NO:58, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:57 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:58, respectively.

[0015] In another example, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:59 and a light chain variable domain related to SEQ ID NO:60, For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:59, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:109), CDR2 (SEQ ID NO:110), and CDR3 (SEQ ID NO:111) sequences of SEQ ID NO:59. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:60, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:112), CDR2 (SEQ ID NO:113), and CDR3 (SEQ ID NO:114) sequences of SEQ ID NO:60.

[0016] The first antigen-binding site, which binds to NKG2D, in some examples, can incorporate a heavy chain variable domain related to SEQ ID NO:61 and a light chain variable domain related to SEQ ID NO:62. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:61, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:63), CDR2 (SEQ ID NO:64), and CDR3 (SEQ ID NO:65) sequences of SEQ ID NO:61. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:62, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:66), CDR2 (SEQ ID NO:67), and CDR3 (SEQ ID NO:68) sequences of SEQ ID NO:62.

[0017] In some examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:69 and a light chain variable domain related to SEQ ID NO:70. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:69, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73) sequences of SEQ ID NO:69. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:70, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:74), CDR2 (SEQ ID NO:75), and CDR3 (SEQ ID NO:76) sequences of SEQ ID NO:70.

[0018] In some examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:77 and a light chain variable domain related to SEQ ID NO:78. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:77, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) sequences of SEQ ID NO:77. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:78, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:82), CDR2 (SEQ ID NO:83), and CDR3 (SEQ ID NO:84) sequences of SEQ ID NO:78.

[0019] In some examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:85 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:85, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:89) sequences of SEQ ID NO:85. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92) sequences of SEQ ID NO:86.

[0020] In some examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:93 and a light chain variable domain related to SEQ ID NO:94. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:93, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:97) sequences of SEQ ID NO:93. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:94, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:98), CDR2 (SEQ ID NO:99), and CDR3 (SEQ ID NO:100) sequences of SEQ ID NO:94.

[0021] In some examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:101 and a light chain variable domain related to SEQ ID NO:102, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:101 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:102, respectively.

[0022] In some examples, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:103 and a light chain variable domain related to SEQ ID NO:104, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:103 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:104, respectively.

[0023] In some examples, the second antigen-binding site can bind to CLEC12A and can incorporate a heavy chain variable domain related to SEQ ID NO:115 and a light chain variable domain related to SEQ ID NO:119. For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:115, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:116), CDR2 (SEQ ID NO:117), and CDR3 (SEQ ID NO:118) sequences of SEQ ID NO:115. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:119, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:120), CDR2 (SEQ ID NO:121), and CDR3 (SEQ ID NO:122) sequences of SEQ ID NO:119.

[0024] In some examples, the second antigen binding site incorporates a light chain variable domain having an amino acid sequence identical to the amino acid sequence of the light chain variable domain present in the first antigen binding site.

[0025] In some examples, the protein incorporates a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains, and/or amino acid sequences at least 90% identical to amino acid sequence 234-332 of a human IgG antibody.

[0026] Formulations containing one of these proteins; cells containing one or more nucleic acids expressing these proteins, and methods of enhancing tumor cell death using these proteins are also provided.

[0027] Another example of the disclosure provides a method of treating cancer in a patient. The method comprises administering to a patient in need thereof a therapeutically effective amount of the multi-specific binding protein described herein. Exemplary cancers for treatment using the multi-specific binding proteins include, for example, acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), myeloproliferative neoplasms (MPNs), lymphoma, non-Hodgkin lymphomas, and classical Hodgkin lymphoma.

[0028] In certain examples, the cancer to be treated is AML selected from undifferentiated acute myeloblastic leukemia, acute myeloblastic leukemia with minimal maturation, acute myeloblastic leukemia with maturation, acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, acute myelomonocytic leukemia with eosinophilia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia (AMKL), acute basophilic leukemia, acute panmyelosis with fibrosis, and blastic plasmacytoid dendritic cell neoplasm (BPDCN).

[0029] In certain examples of the present disclosure, the cancer is MDS selected from MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD), MDS with ring sideroblasts (MDS-RS), MDS with excess blasts (MDS-EB), MDS with isolated del(5q), and MDS, unclassified (MDS-U).

[0030] In certain examples of the present disclosure, the ALL to be treated is selected from B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL). In examples of the present disclosure, the MPN to be treated is selected from polycythaemia vera, essential thrombocythemia (ET), and myelofibrosis. In certain examples of the present disclosure, the non-Hodgkin lymphoma to be treated is selected from B-cell lymphoma and T-cell lymphoma. In certain examples of the present disclosure, the lymphoma to be treated is selected from chronic lymphocytic leukemia (CLL), lymphoblastic lymphoma (LPL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), follicular

lymphoma, mantle cell lymphoma, hairy cell leukemia, plasma cell myeloma (PCM) or multiple myeloma (MM), mature T/NK neoplasms, and histiocytic neoplasms.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] **FIG. 1** is a representation of a heterodimeric, multi-specific antibody (a trispecific binding protein (TriNKET)). Each arm can represent either the NKG2D-binding domain, or the tumor associated antigen-binding domain. In some embodiments, the NKG2D- and the tumor associated antigen- binding domains can share a common light chain.

[0032] **FIG. 2** is a representation of a heterodimeric, multi-specific antibody. Either the NKG2D-binding domain or the tumor associated antigen-binding domain can take the scFv format (right arm).

[0033] **FIG. 3** are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to human recombinant NKG2D in an ELISA assay.

[0034] **FIG. 4** are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to cynomolgus recombinant NKG2D in an ELISA assay.

Claims

1. A protein comprising:

(a) a first antigen-binding site that binds NKG2D;

(b) a second antigen-binding site that binds CLL-1/CLEC12A; and

(c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

2. The protein of claim 1, wherein the first antigen-binding site binds to human and non-human primate NKG2D.

3. The protein of claim 1 or 2, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

4. The protein according to claim 3, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.

5. The protein according to claims 3 or 4, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

6. The protein according to claim 5, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

7. The protein according to claim 5 or 6, wherein the light chain variable domain of the first antigen-binding site has an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.

8. The protein according to any one of the preceding claims, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to an amino acid sequence selected from: SEQ ID NO:85, SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, and SEQ ID NO:93.

9. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID

NO:85 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:86.

10. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:41 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:42.

11. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:49 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:50.

12. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:57 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:58.

13. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:59 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:60.

14. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:61 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:62.

15. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:69 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:70.

16. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:77 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:78.

17. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:93 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:94.

18. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:101 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:102.

19. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises a heavy chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:103 and a light chain variable domain amino acid sequence at least 90% identical to SEQ ID NO:104.

20. The protein according to any one of claims 1-8, wherein the first antigen-binding site comprises:

(i) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:43, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:44, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:45, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:46, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:47, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:48;

(ii) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:51, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:52, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:53, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:54, a light chain CDR2

sequence identical to the amino acid sequence of SEQ ID NO:55, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:56;

(iii) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:109, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:110, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:111, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:112, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:113, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:114;

(iv) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:63, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:64, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:65, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:66, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:67, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:68;

(v) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:71, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:72, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:73, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:74, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:75, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:76;

(vi) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:79, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:80, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:81, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:82, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:83, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:84;

(vii) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:87, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:88, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:89, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:90, a light chain CDR2

sequence identical to the amino acid sequence of SEQ ID NO:91, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:92; or

(viii) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:95, a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:96, a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:97, a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:98, a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:99, and a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:100.

21. The protein of claim 1 or 2, wherein the first antigen-binding site is a single-domain antibody.

22. The protein of claim 21, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

23. The protein according to any one of claims 1-2 or 21-22, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

24. The protein of claim 23, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

25. The protein according to any one of claims 1-4 or 8-20, wherein the second antigen-binding site is a single-domain antibody.

26. The protein of claim 25, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.

27. The protein of any one of claims 1-26, wherein the second antigen-binding site comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:115 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:119.

28. The protein of claim 27, wherein the second antigen-binding site comprises:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:116;

a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:117; and
a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:118.

29. The protein of claim 28, wherein the second antigen-binding site comprises:
a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:120;
a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:121; and
a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:122.

30. The protein according to any one of claims 1-29, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains.

31. The protein according to claim 30, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.

32. The protein of claim 30 or 31, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

33. The protein according to any one of claims 30-32, wherein the Fc domain comprises an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

34. A formulation comprising the protein according to any one of the preceding claims and a pharmaceutically acceptable carrier.

35. A cell comprising one or more nucleic acids encoding the protein according to any one of claims 1-33.

36. An *in vitro* or *ex vivo* method of directly and/or indirectly enhancing tumor cell death comprising exposing the tumor cell and a natural killer cell to an effective amount of the protein according to any one of claims 1-33.

37. Use of the protein according to any one of claims 1-33 or the formulation according to claim 34 in the manufacture of a medicament for treating cancer.

38. The use of claim 37, wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), myeloproliferative neoplasms (MPNs), lymphoma, non-Hodgkin lymphomas, and classical Hodgkin lymphoma.

39. The use of claim 38, wherein the AML is selected from undifferentiated acute myeloblastic leukemia, acute myeloblastic leukemia with minimal maturation, acute myeloblastic leukemia with maturation, acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, acute myelomonocytic leukemia with eosinophilia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia (AMKL), acute basophilic leukemia, acute panmyelosis with fibrosis, and blastic plasmacytoid dendritic cell neoplasm (BPDCN).

40. The use of claim 38 or 39, wherein the AML is characterized by expression of CLL-1 on the AML leukemia stem cells (LSCs).

41. The use of claim 40, wherein the LSCs further express a membrane marker selected from CD34, CD38, CD123, TIM3, CD25, CD32, and CD96.

42. The use of any one of claims 38-41, wherein the AML is a minimal residual disease (MRD).

43. The use of claim 42, wherein the MRD is characterized by the presence or absence of a mutation selected from *FLT3-ITD* ((Fms-like tyrosine kinase 3)-internal tandem duplications (ITD)), *NPM1* (Nucleophosmin 1), *DNMT3A* (DNA methyltransferase gene *DNMT3A)*, and *IDH* (Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2)).

44. The use of claim 38, wherein the MDS is selected from MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD), MDS with ring sideroblasts (MDS-RS), MDS with excess blasts (MDS-EB), MDS with isolated del(5q), and MDS, unclassified (MDS-U).

45. The use of claim 38, wherein the MDS is a primary MDS or a secondary MDS.

46. The use of claim 38, wherein the ALL is selected from B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL).

47. The use of claim 38, wherein the MPN is selected from polycythaemia vera, essential thrombocythemia (ET), and myelofibrosis.

48. The use of claim 38, wherein the non-Hodgkin lymphoma is selected from B-cell lymphoma and T-cell lymphoma.

49. The use of claim 38, wherein the lymphoma is selected from chronic lymphocytic leukemia (CLL), lymphoblastic lymphoma (LPL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), follicular lymphoma, mantle cell lymphoma, hairy cell leukemia, plasma cell myeloma (PCM) or multiple myeloma (MM), mature T/NK neoplasms, and histiocytic neoplasms.

50. The protein according to any one of claims 1-33 or the formulation according to claim 34 for use in therapy.